*Isolation of* **1**. Fresh leaves and stem (550 g) of *Phalaenopsis* cv musashino (cryde × maliburiver) were homogenized with MeOH and stored at room temp overnight The MeOH extract (FR 1; 12 g) was suspended with 0 1 M HCl (100 ml, pH 2–3) and extracted with $Et_2O$(Fr 2, 1 23 g) and EtOAc(Fr 3, 0 46 g), successively. The aq layer was adjusted to pH 9 with $NH_3$ and extracted with $CHCl_3$ (Fr. 4, 0 24 g) and *n*-BuOH (Fr 5, 1 02 g), successively. The Fr 4 was repeatedly subjected to column chromatography to give a pale yellow powder (140 mg) which gave colourless crystalline **1** after recrystallization from EtOH Mp 99–104° (lit. [5], mp 104 5–105°), $[\alpha]_D^{22} - 24\ 5$ ($CHCl_3$, *c* 0 57), (lit [5], $[\alpha]_D^{20} - 15°$), FDMS *m/z* 361 ($M^+$), $^1$H NMR ($CDCl_3$) 2 76, 3 04 (2H, *dd*, $J = 16$ Hz), 2 98 (2H, *d*, $J = 1\ 2$ Hz), 3.65 (3H, *s*), 3 99, 4 20 (2H, *ddd*, $J = 6$, 6, 11 Hz), 7.25 (5H, *s*), $^{13}$C NMR ($CDCl_3$). 26 0, 30 6, 31 8, 42 9, 44 8, 45 5, 51 8, 54 4, 54 8, 67 9, 68 3, 75 8, 127 2, 128 2, 130 2, 134 8, 171 0, 174 2

*Acknowledgement*—We wish to thank Miss S Takeda for her technical assistance We are grateful to Mr K Yamashita for donation of *Phalaenopsis* spp Thanks are due to Mr Y Tanaka, Miss Y Soeda and Mr R Isobe for $^1$H NMR, $^{13}$C NMR, FDMS analysis

## REFERENCES

1 Ishii, M, Shoyama, Y, Uemoto, S, Nishioka, I and Fujieda, K. (1976) *Sci Bull Fac Agric Kyushu Univ* **31**, 99
2 Ishii, M, Uemoto, S, Fujieda, K, Nonaka M, Shoyama, Y and Nishioka, I (1976) *Phytochemistry* **18**, 1211
3 Tai, C S, Uemoto, S, Shoyama, Y and Nishioka, I (1981) *Phytochemistry* **20**, 2565
4 Shoyama, Y, Hatano, K, Nishioka, I and Yamagishi, T (1987) *Phytochemistry* **26**, 2965
5 Brandange, S and Lunning, B (1969) *Acta Chem Scand* **23**, 1151
6 Gohbara, M, Kosuge, Y, Yamasaki, S, Kumura, Y, Suzuki, A and Tamura, S (1978) *Agric Biol Chem* **42**, 1037

*Phytochemistry*, Vol. 27, No 5, pp 1566 1567, 1988
Printed in Great Britain

0031 9422/88 $3 00 + 0 00
Pergamon Press plc

# DISTRIBUTION OF PIPERINE IN VEGETATIVE PARTS OF *PIPER NIGRUM*

URSULA SEMLER and GEORG G GROSS*

Universitat Ulm, Abteilung Allgemeine Botanik, Oberer Eselsberg, D-7900 Ulm, F R G

(*Received* 7 *August* 1987)

**Key Word Index**—*Piper nigrum*, Piperaceae, pepper; piperine, high performance liquid chromatography

**Abstract**—Piperine contents of greenhouse-grown *Piper nigrum* plants were analysed Only traces of alkaloid were detected in leaves and young shoots, more significant quantities (*ca* 0 03%) occurred in the root Remarkably high amounts of piperine (*ca* 0 2%) were found, however, in mature, fully differentiated shoots reaching the contents of commercially available white or black pepper of lower quality

Piperine (*E,E*-1-*N*-piperoylpiperidine), the pungent principle of pepper, occurs in the fruit of *Piper nigrum* and related species, e g *P longum* or *P clusii* [1] This alkaloid has also been found occasionally in other parts of piperaceous plants, e.g in stems of *P chaba* [2] and in the wood of *P novae-hollandiae* [3]. In general, however, the association of pepper seeds and piperine is so characteristic that it is widely believed that vegetative parts of pepper are devoid of this substance

Our interest in this question arose from plans to study the biogenesis of piperine. We soon became aware that it was extremely difficult, if not impossible, to achieve flowering and fruiting of *P nigrum* plants in the greenhouse at our northern geographical latitudes It was thus decided to analyse vegetative plant parts for the eventual accumulation of piperine. For this purpose, roots, young and mature shoot segments, and young and mature leaves, respectively, were extracted individually with $CHCl_3$ In addition, commercially available samples of black and white pepper kernels were worked up as references Qualitative analyses by reversed-phase HPLC, based on comparison of $R_f$s and spiking with authentic material, revealed that the extracts from root and shoot tissues contained significant amounts of piperine as the main or almost exclusive component Extracts from mature shoots in particular closely resembled those

*Author to whom correspondence should be addressed

from pepper seeds. In contrast, only minute traces of piperine, together with high concentrations of unidentified compounds, were detectable in the leaf extracts

These results were confirmed by quantitative studies (Table 1) leading to the completely unexpected finding that piperine concentrations in mature shoots of *P. nigrum* almost reached those from commercial pepper samples of minor quality It is interesting to mention in this context that the dried shoots even developed the characteristic odour of fresh pepper upon grinding.

In further experiments, piperine from extracts of mature shoots was isolated by semi-preparative HPLC. The purified product co-chromatographed with authentic piperine upon TLC, and its nature was further substantiated by EIMS (see Experimental). Thus, there is no doubt that vegetative parts of *P. nigrum* plants are also capable of synthesizing piperine in appreciable amounts and can be employed for biosynthetic studies. We are currently concerned with experiments on this latter aspect, in particular on the question of the eventual participation of piperoyl-CoA [4] as an activated intermediate in this process

Table 1 Distribution of piperine in different parts of *Piper nigrum* plants

| Plant material* | Piperine (mg/g fr. wt) | (mg/g dry wt) |
|---|---|---|
| Root | 0.057 | 0 32 |
| Leaves· young | 0 0006 | 0.0049 |
| mature | 0 0002 | 0 0007 |
| Shoot. young | 0.0087 | 0 070 |
| mature | 0 46 | 1 92 |
| Fruit: black pepper, | | |
| sample 1 | — | 2.88 |
| sample 2 | — | 42 0 |
| sample 3 | — | 17.3 |
| white pepper | — | 2 25 |

*Origin and definition are described in Experimental

## EXPERIMENTAL

*Plant material P nigrum* was grown in the greenhouse (day/night temp · 22/19°) For the expts, 2- to 4-year-old plants were divided into young shoots (41 g fr. wt) and leaves (58 g fr. wt, first 15–20 cm from shoot tip), mature shoots (77 g fr wt) and leaves (110 g fr wt, from fully differentiated, lignified shoots), and roots (35 g fr wt) Samples of commercial white and black pepper were obtained from local stores and pharmacies.

Plant parts were dried overnight at 80°, finely powdered and Soxhlet extracted with EtOH for 3 hr This soln was evapd *in vacuo*, the residue taken up in 50 ml $H_2O$, basified with $NH_4OH$ and extracted with $4 \times 50$ ml $CHCl_3$ The organic phase was dried ($CaSO_4$), evapd and the residue taken up in little THF All steps were carried out in the dark to avoid photoisomerization of piperine

*Analytical procedures* Conditions for analytical HPLC were LiChrosorb RP-18 (Merck), particle size 5 μm, column 180 × 3 mm i d , flow rate 1 ml/min, solvent [5] $H_2O$–MeCN–THF (69·32 8), detection UV 345 nm For semi-preparative sepns a Merck LiChrosorb RP-18 column (250 × 7 mm i d.), 7 μm particle size, was used with the same solvent (flow rate 5 ml/min) Quantitative determinations were made with a computing integrator using pure piperine (Aldrich) as ext std Identity and purity of piperine isolated from plant material was checked by TLC on silica gel G with toluene–EtOAc (7·3) and by EIMS (70 eV), *m/z* 285 $[M]^+$ (91%), 201 $[M-C_5H_{10}N]^+$ (100), 173 (63), 143 (35), 115 (70)

*Acknowledgements*—We thank Dr G Schmidtberg (Ulm) for recording and interpretation of MS and the Fond der Chemischen Industrie for financial support

## REFERENCES

1 Marion, L. (1950) in *The Alkaloids. Chemistry and Physiology* (Manske, R H. F. and Holmes, H. L, eds) Vol I,, p 165 Academic Press, New York
2 Mishra, S S and Tewari, J P (1964) *J. Pharm Sci* **53**, 1423
3 Loder, J. W , Moorhouse, A. and Russell, G B (1969) *Aust J. Chem* **22**, 1531
4. Semler, U , Schmidtberg, G and Gross, G G (1987) *Z. Naturforsch* **42C**, 1070
5. Archer, A W (1986) *J Chromatogr* **351**, 595